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Nitrogen and Phosphorus Analysis in Field Cultivation of Pak choi

– effect of two different compositions of fertilisers on plant and soil nutrient status

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Nitrogen and Phosphorus Analysis in Field Cultivation of Pak Choi

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Kväve- och Fosforanalys i Fältodling av Pak Choi

- effekt av två olika sammansättningar av gödselmedel på växt- och jordnäringsstatus

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Abstract

This pilot study was implemented to reflect the delivery and the plant availability of nitrogen and phosphorus in response to different compositions of fertilisers approved for organic farming in Sweden. The experimental approach was to compare the concentration of nitrogen and phosphorus in plant sap of Pak choi plants grown in soil treated with different organic waste such as aged cattle manure and a liquid retting digest derived from the biogas industry, with plants grown in soil treated with a blend of pelleted organic fertilisers derived from the Swedish slaughterhouse industry.

The organic waste materials and fertilisers in this study were selected with respect to their nutritional properties: concentration of macro elements and the assumed nitrogen accessibility from the organic and inorganic proportion of nitrogen present in these materials. All plant tissues and soil samples were analysed for: the nutritional status in soil (all macro nutrients) prior to fertiliser application as well as the concentration of nitrogen and phosphorus in soil postharvest; plant sap concentration of nitrogen and phosphorus on three occasions along six weeks of field cultivation and the concentration of young leaf tissue total nitrogen concentration on same occasions. In addition, three days prior to final harvest, the treatments were sampled to measure fresh and dry weight of leaves and roots followed by a subsequent analysis of total nitrogen accumulation in the same tissues.

Plant sap concentration of inorganic nitrogen was highest in tissues sampled from Pak choi plants grown in soil treated with pelleted slaughterhouse waste in week four, the second occasion of sampling. This concentration decreased to the lowest relative to the concentration of plants grown in control soil (no fertilisers) and plants grown in soil treated with aged cattle manure and retting digestate in week five, which was the last occasion of sampling.

Soil remaining concentration of inorganic nitrogen postharvest was shown to be the highest in soil treated with pelleted fertilisers but the leaf tissue concentration of total nitrogen showed the lowest concentrations in plants sampled for dry weight grown in the pelleted fertiliser treatment. An opposite pattern was found in soil treated with aged cattle manure and retting digestate which is contradictive and further discussed.

Plant sap concentration of phosphorus showed the highest values for control plants, surprisingly during all three occasions of sampling. This relationship indicated that the consumption of phosphorus can be limited by a relative low concentration of other macro elements, *in planta*, for the plants grown in the control soil. Moreover, postharvest soil remaining phosphorus indicated redundancy in soil treated with aged cattle manure and retting digestate, because the plant sap concentration of phosphorus (of plants grown in control soil) where similar to the plant sap concentrations of plants grown in soil treated with the pelleted fertilisers. The initial amount of added phosphorus were three times less in the soil treated with pelleted fertilisers, in comparison to the soil treated with aged cattle manure and retting digestate.

Content

Introduction

Field studies of organic fertilisers including market products and industrial waste materials are important in order to establish guidelines for efficient utilisation in organic and conventional/integrated plant production. The Swedish organic agriculture land area has increased in proportion to the total Swedish agriculture land area by approx. 15% from 2005 – 2019 (SCB 2019). In 2019, fractions of the total agriculture land area, classed as organic arable land, covered approx. 19% of total Swedish arable land (SCB 2019). Expansion of organic farmland indicates increased usage of organic fertilisers and manure which is a reason to apply research for sustainable nutrient utilisation of these materials in organic plant production.

Several types of organic waste such as excrements, urine, bone-, meat-, and blood-meal generated from the livestock industry is currently utilised as fertilisers and manure in Swedish plant production. Industrial waste partially consists of plant macro elements: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S). These elements are (beyond carbon, oxygen and hydrogen) essential for plant growth, development and reproduction. Deficient levels of such elements in plants will cause yield loss and lower the quality of produce. Further, microelements (iron, manganese, boron, copper, zinc, molybdenum) play different roles in general plant metabolism as well as in plant defence (Dordas 2008); deficient levels of microelements will affect the status of growth and quality of produce.

The concept of conventional/integrated (i.e. non-organic) plant production is given to distinguish organic plant production from conventional systems in the following text. Conventional plant producers have the ability to optimise their fertiliser regimes due to a broader assortment of different products containing crop specific concentrations of plant growth essential elements, or by compiling customised concentrations for each crop. On the other hand, organic producers are relatively restricted when it comes to crop specific fertilisers, however, the assortment of processed products expands on the market. Farmyard manure, industrial waste and/or any other soil amendments suited for application as fertilisers craves some experience to gain as much as possible of their nutritional capacity. For instance, some elements can be overrepresented for the plant while others are present in relative low concentrations when it comes to industrial waste. Moreover, organic fertilisers and waste materials are allowed in conventional plant production but conventional products are prohibited in organic production. Inappropriate application of different industrial waste materials and farmyard manure affects the environment around arable land regardless of the production system (conventional or organic). In this study, some of the most common waste materials and manure will be presented and assayed.

Aged cattle manure (ACM)

Aged cattle manure is composed of cattle excrements/urine, straw and hay. The straw and hay are added frequently to the flooring in barns in order to mix with droppings from the animals which are kept in the barns during the winters. This mixture can be stacked in piles and covered to reduce losses of N through gasification. These piles can be aged (decomposed) over the summer season and winter, followed by field application in spring the year after. This strategy of manure recycling with subsequent field application is important to maintain soil fertility and to avoid soil depletion of organic matter in both organic and conventional crop production. Manure application supplies the soil with macro/micro elements and enriches the concentration of carbon-containing molecules.

Pelleted slaughterhouse waste (PSW)

Organic waste derived from the slaughter industry can be compressed to pellets and applied as fertilisers in organic plant production. These pellets can be composed of both slaughterhouse waste and farmyard manure to balance their nutritional proportions which broadens the product for a wider range of crop specific application. Pelleted slaughterhouse waste can be used by organic plant producers that lacks animal holding and other sources of materials used as organic fertilisers. However, the content of carbon is relatively low in such products. Pelleted fertilisers are, in general, comprehensive in nutritional composition since they are composed of mixed materials and uniform in size which simplifies application.

Biogas retting digestate (BRD)

Retted plant- and animal-based digestate has a high concentration of (inorganic) ammonium-N. The high ammonium-N concentration is due to the fact that most of the carbon (C) is released as carbon dioxide and methane during the retting process. This process lowers the ratio of C to N (C/N) since the relative N concentration increases in the BRD as a result from the gasification of carbon. The BRD is further separated into two different fraction sizes, however, the dry substance in BRD is very low (< 3%) which means that most of the N-content is dissolved in the solution. This fertiliser is a rapid source of ammonium-N and approved for application in organic plant production, but there are some varieties of BRD that are not approved for organic crop production depending on the origin of the input material.

Soil organic matter (SOM)

In this thesis soil organic matter (SOM) is used in following context: organic matter associated with human inputs to maintain target levels of carbon and nutrients in arable land. Organically bound N can be released to soil from SOM as inorganic N through a process called mineralisation, a microbial activity in which carbon bound N like amino acids (AA) is converted to ammonium N. The C/N ratio of different soil amendments impacts N mineralisation, where materials with high C/N releases N slowly because the proportion of carbon is relatively high compared to the proportion of N. Moreover, available soil N is partly targeted for microbial consumption which is crucial to maintain further decomposition of SOM with continuous release of N. The microbial sequestration of soil N is named immobilisation which means that the inorganic-N is fixed back into organic forms, i.e. to serve microbial metabolism. Plants and microorganisms compete for soil N.

Inorganic N (ammonium and nitrate) is just a part of all plant absorbable forms of N in soil because organic molecules of N like AA and peptides are also absorbable by plant roots. For instance, organic forms of N have shown to be absorbable by 'Bok choy' roots (Watanabe *et al*. 2012); 'Bok choy' is another name for Pak choi (*Brassica rapa subsp. chinensis* L.H. Bailey). Moreover, *Arabidopsis thaliana* a model plant for research which belongs to the same family as Pak choi (*Brassicaceae*) was able to grow/develop receiving AA as the only source of N (Hirner *et al.* 2006). Mutant plants for the AA transporter Lysine Histidine Transporter1 (LHT1) which is the protein that mediates AA uptake in roots failed to absorb AA (Hirner *et al.* 2006). Furthermore, roots of *A. thaliana* have shown a 6 to 10-fold increase of AA uptake, over nitrate-N, when both nitrate-N and AA were present simultaneously (Jämtgård 2010). This uptake ratio was shown to depend on 24 h root pre-exposure to AA indicating that *A. thaliana* adapts N absorption to N conditions in soil (Jämtgård 2010).

Retting digests, farmyard manure and other sources of soil amendments enriches soil with organic acids, humus and different mono- and polymers (SOM) in addition to the plant absorbable macro and micro elements. Deng *et al*. (2019) showed that addition of a commercial retting solution to soil increased the Shannon Diversity (a mathematical function used to characterise species diversity in a community) of bacterial species *inside* the roots of strawberries, compared to the species diversity found in soil and rhizosphere. Diverse soil microbial communities have been discussed to suppress soil borne pathogens to a higher extent than communities with less diversity (Fukui *et al*. 2003). The abundancy and diversity of soil bacterial communities are affected by different soil parameters, including pH and soil carbon levels (Liao *et al.* 2018; Lauber *et al.* 2009); inputs of SOM alter the given parameters. Soil microorganisms generate important compounds which react with plant elements in a molecular process called chelating (Ahmed 2015). Microbial secretion of natural chelating agents (siderophores) enable uptake of different microelements by forming complexes which are absorbable by plant roots. Iron is a microelement which chelates with siderophores produced by both bacterial and plant species (Albelda-Berenguer *et al.* 2019). In summary, the diversity of soil microbes can be altered depending on man-made inputs of SOM which can affect the suppression of soilborne pathogens as well as promoting soil fertility in several ways.

Fertiliser management & soil properties

Soil organic matter promote soil with important molecules, ions, structure and aeration. Plant cells require oxygen (O_2) for aerobic metabolism driven by respiration and ATP biosynthesis specially to maintain stable absorption of macro and micro elements into roots. This has been demonstrated in an experiment where waterlogging of spring wheat and barley caused lowered absorption of macro and micro elements, revealed by measuring the concentration of these elements in shoot tissues under stress conditions (Steffens *et al.* 2005). Soil physical condition determines the level of drainage which is associated with O_2 mobilisation in the top soil layers.

In modern agriculture, a sustainable approach to decrease eutrophication in watercourses, lakes and oceans is to make a batch specific nutritional analysis of any material prior to soil application. Plug plants of leafy vegetables demand relatively high levels of nutrition (for yield maximisation) instantly in connection to field transplantation while others like trees, shrubs and directly seeded plants cultivated for fruits can be slightly different in the growth-stage dependent nutritional demand for fruit maximisation. The plant available elements present in a specific material do not necessarily correspond to the demand of a certain crop. The material specific rate of nitrogen release through mineralisation will alter the proportions of inorganically- to organically-bound N which determines the accessibility of N for the plant through the growing season. Hirner *et al.* (2006) showed that the LHT1 is present in the mesophyll cells where they assist AA uptake. The mesophyll cells are located in the leaves and the AA can be transported in vascular tissues by other transporter proteins, indicating a long-distance transport from the roots to the leaves. In fact, the LHT1 protein has shown to be expressed in every tissue of *A. thaliana* (Chen & Bush 1997). The absorption property of AA is not equal for all plant species. Different plant species show different traits in N absorption depending on root specific properties, which is a species dependent adaptation – a result of plant evolution in different environments. For instance, the study performed by Watanabe *et al.* (2012) showed that roots of Bok choy plants can absorb a significant higher proportion of organic N when present together with ammonium-sulphate, in comparison to tomato plants. The results in Watanabe *et al.* (2012) indicate that the levels and forms of N (organic and inorganic) is important to consider for efficient species dependent fertiliser management in agriculture since different plant species exhibits *different adaptations* of N absorption.

Appropriate fertiliser management decrease the loss of N from field soil to the atmosphere. For instance, the proportion of ammonium to ammonia is partially driven by the soil pH, where alkaline soils (pH > 7) risks the ammonium (NH_4) to lose a proton and become ammonia (NH_3). Ammonia can be lost through a process called ammonia volatilisation which implies loss of ammonia from the soil to the atmosphere, especially if the ammonia-N is present in the surface of the topsoil where wind is present. Moreover, nitrification (a series of microbial activity) can turn ammonium-N into nitrate-N (NO_3^-), which is another form of plant available inorganic-N and this process is affected by the levels of moisture, pH and O_2 present in the soil. Furthermore, the nitrate-N can get lost through a sequence of microbial processes called denitrification, in which nitrate-N is converted to nitrogen gas (N_2) as well as other gaseous forms of N (like NO and N_2O) and emitted to the atmosphere (Schlüter *et al.* 2018).

In brief, soil fertility is a concept of several factors: soil pH, soil temperature, the proportion of negatively charged clay particles (called soil colloids), the concentration of different elements present per volume of soil, the level of moisture in the soil which alters the concentration of dissolved elements and the soil flow of O_2; soil porosity determines the flow of O_2 and the water holding capacity. Inputs of SOM in soil alters the proportion of soil macro and micro pores. If a clay soil low in SOM is completely dried, dense clods will be formed as a consequence of tillage, and soil cracking will appear during the season.

The size and composition of soil particles determines the physical and chemical properties in different soil types. Clay rich soils can hold cations and water to a higher extent compared to sandy soils. Clay soils attract ammonium-N to a higher extent than sandy soils; sandy soils drain water to a higher extent than clay soils. This means that clay soils are less sensitive to percolation of dissolved N and P. On the other hand, sandy soils dry out faster and have larger pores which enables efficient penetration of O_2 (oxygenation). However, regardless of soil particle size nitrate and nitrite (both anions) do not add to negatively charged soil colloids and for this reason negatively charged N as well as P compounds are sensitive to percolation/leaching since phosphates are also anions. Since loam and clay soils hold water to a higher extent than sandy soils, anions are relatively less subjected to percolation and leaching.

Aim

The aim of this work was to investigate the nutrient delivery of N and P from different fertilisers in two nutritionally balanced treatments by analysing the difference of plant accumulated total N and plant sap levels of N and P in plants grown in these treatments. Pak choi was assayed to mirror the nutritional delivery from the treatments through sampling of plant tissues and analysis of plant sap. Soil samples were taken postharvest to determine soil remaining levels of N and P. This work is dedicated to a growing segment of young farmers that needs to understand the properties of different fertilisers and soil amendments in order to practice appropriate fertiliser management. Compositions and forms of nutrient rich organic matter can be optimised to treat growth stage dependent crops with the right concentration at the right time of application. Since materials release nutrients at different rates composition is crucial to achieve precision in application.

Hypothesis

Pak choi was grown in two different compositions of fertilisers approved for organic plant production, in replicated experimental units (plots). The details of each treatment are described in material and methods. Null hypothesis will be true if elements are balanced properly according to assumed release rates.

Null hypothesis 1 & 2

$H_{0(1)}$: There will be no statistical difference between estimated mean values of above ground harvest (the canopy) measured on plants grown in the nutritionally balanced treatments of fertilisers.

$H_{0(2)}$: There will be no statistical difference between estimated mean values of fresh weight and dry weight of leaves and roots sampled from the treatments prior to the canopy harvest - described in $H_{0(1)}$.

Limitations

The individual plants in each treatment were most likely affected by the heterogeneity of soil nutrition (i.e. variation of macro elements in each plot) and other random biotic and abiotic factors such as pests, differences in physical and chemical soil properties, edge effects etc. For this reason, it is important to spread the errors as much as possible by using many replicated plots for each treatment in a total randomised experimental design (without blocks).

Drip irrigation was installed to provide a similar supply of water in each plot but differences in soil structure may affect the water holding capacity in the soil of each treatment, since incorporation of different materials in the soil affects soil structure on treatments specific level.

Precipitation was not monitored, however, soil moisture at two depths were monitored to reduce flushing of anions/nutrients to deeper soil layers. All nutritional analysis data will be based on pooled samples from each treatment.

The pooling method returns a single value of each element, within each treatment, and the drawback here is that no statistical variance can be predicted from the underlying samples in the pool. Pooling will return a good approximation of different parameters from the replicated plants in each treatment.

Several greenhouse studies under controlled conditions can be conducted prior to a study like this, to develop references of plant nutrient levels in controlled environments. This can help with interpreting the results of nutrient concentrations obtained at field conditions. On the other hand, the drawback with greenhouse studies is that the field soil is only present at the field, not in the greenhouse. Peat is not the same as field soil. Abiotic and biotic factors can be mimicked close to field conditions and field soil can be transferred to a closed system for running environmentally controlled assays. However, the field will always be the most accurate environment to consider for comprehensive studies to optimise field grown crops.

Material and methods

This experiment was conducted in the south of Sweden at the property of SLU in Alnarp. The fertilisers described in the introduction (BRD, ACM and PSW) were tested for nutrient delivery in this project. They contained both inorganic-N and organic-N in different proportions. Their relative contribution of N in each treatment was computed with respect to their proportional amount of organic-N. An estimation was made of the amount of N that could be available from the proportion of organic-N in BRD, ACM and PSW:
10% N of the proportion of organic-N in ACM were estimated to be plant available (Sullivan 2020, see table 2 for dairy cattle compost*); 75% N of the proportion of organic-N in PSW were estimated to be plant available* (SJV 2019)*; BRD was assumed to have its total-N content (100%) available in connection to application* (SJV 2016).
Liquid ammonium containing fertilisers, like the BRD, transports the ammonium for some depth into the soil. Post application of water through irrigation or from precipitation lowers the exposure of ammonium to air exchange. This study selected two PSW products based on Pak choi (in fact Chinese cabbage) nutritional recommendations which was obtained from agricultural advisors. All treatments in this experiment are listed below:

T0. Null treatment (the control) without fertilisers + tillage

T1. PSW + additional fertilisers + tillage

T2. BRD + ACM + additional fertilisers + tillage

Field location & soil conditions & soil tillage

Treatment 1, treatment 2 and treatment 3 were assayed in a loam soil (19% clay content) located in zone 1 (Latitude 55.661073191301064 N, Longitude 13.078488124569668 E). The field had been in fallow for three years (2017, 2018, 2019) prior to this experiment and it held an organic content of < 3%. In 2016 (the last year of cultivation in this field site), field beans and spring-wheat were intercropped.

The soil was tilled with a rotary cultivator at a depth of approx. 15 cm, one week prior to fertiliser application and field transplantation of Pak choi plug plants (seedlings having three to four developed true leaves). This tillage was done mostly to loosen the soil and disturb weeds; a second tillage was carried out to mix the soil with the fertilisers in connection with fertiliser application.

Division of field area

A total area of 10 m (width) x 20 m (length) = 200 m^2 was set aside for this experiment. A subdivision was made of 10 rows across the length of that area and each row got a width of 1 m and a length of 10 m. Each of these 10 rows was further divided into three experimental units (i.e. plots). Each plot got an area of 2,2 m (length) x 1 m (width) = 2,2 m^2. Margins for spacing were set at 0.5 m on both sides of all plots. In total, 30 plots were established for T1, T2 and T0 - which is 10 plots per treatment.

Soil sampling - initial

The soil was sampled at 30 points (one sample taken from the midpoint of each plot) from a cross section of 20 cm. All these soil samples were pooled (mixed together) and one representative sample of this soil pool was then analysed with a method called the Modified Spurway. The Modified Spurway method reflects the easily soluble plant macro and micro element concentration present in the soil and available for plants over a relatively short period of time; in contrast to an AL-analysis. This pooling method returned a single result representative for the nutritional concentration of macro elements in the field soil. The amount of application of ACM, PSW and BRD were computed with respect to the present concentration of macro elements in soil.

Fertiliser analysis

The ACM and the BRD were used mainly to balance for K and N, respectively, in T2. Prior to computation of fertiliser application, these two waste materials were analysed (method: Kjeldahl+dewardas) for macro element concentrations and with two specifications of N (total-N + ammonium-N). The ACM were further analysed for nitrate-N (method: QuAAtro) because 'Kjeldahl+dewardas' only specifies total-N subdivided into ammonium-N. However, the nitrate-N was necessary to compute the proposition of organic-N in ACM and PSW (free amino acids, peptides, proteins, microbial nucleic acids, urea, etc.).

Ten percent of the total-N content in cattle manure consisted of ammonium-N and the remaining N was assumed to be the amount of organic-N, with a negligible content of nitrate-N. However, the nitrate analysis of ACM (post field application) revealed that the concentrations of nitrate-N and ammonium-N were similar, which means that approx. 20% of the total-N content in that *batch specific* ACM consisted of inorganic N.

For balancing of T1, the nutritional concentrations of two pelleted fertilizers (NPK 6-3-12+7S and N15) were obtained from the packaging of these products, and the concentration of ammonium-N in these products (NPK 6-3-12+7S and N15) were further complemented with analysis data obtained from the PSW producer.

Compiling & adding the fertilisers

The N and K were balanced according to 'Chinese cabbage' specific levels obtained from agriculture advisors, as already mentioned. The intention with balancing was to not underestimate the nutrient demand of macro elements for Pak choi plants and in order to provide similar concentrations of nutrition in both T1 and T2. This was done to prevent the possibility that N and P concentration in plant tissues could be altered by deficient levels of other macro elements. A rapid N fertiliser like the BRD is suited for pre-cultivated seedlings, in terms of rapid N access to promote maximum growth in connection with field transplantation. Most of the log-phase (Pak choi and several plants develop according to an S-curve) has passed during the pre-cultivation of seedlings in the nursery.

All fertilisers (except dolomite stone meal) that were used in T1 were added to the soil and tilled at a depth of approx. 15 cm prior to field transplantation of Pak choi seedlings. All the components (except BRD) of the fertilisers which were used in T2 were also tilled into the soil prior to transplantation of the seedlings. Further, the dolomite meal was added a week past field transplantation of seedlings to balance the magnesium concentration and the BRD were added after some days of precipitation, 7 days past transplantation, to reduce volatilisation of ammonium-N (NH_4^+) to ammonia-N (NH_3). Soil pH = 7,8. Table S1 in supplementary material provides information of added fertilisers and their resulting summary of macro elements in each treatment.

Irrigation & installation of sensors for soil moisture/temperature-measurements

Drip-irrigation was installed ten days post field transplantation. The plots were watered (all with the same volume and procedure) by hand prior to the installation of drip-irrigation. Soil moisture was kept at low fluctuation with the assistance of sensors which measured the moisture (or soil pore under-pressure) at two depths: 10 and 30 cm. Precipitation was not monitored. Specific changes in soil elements concentration affected by altered water levels caused by rain were not of interest. The drop-irrigation was installed to maintain *similar* soil moisture levels in all treatments to avoid general plant stress caused by drought events.

Soil & plant tissue sampling

All samples of plant tissues and soil which were analysed for macro element concentrations were pooled in this study. Pooling is usually suited for different DNA and RNA studies when many samples are taken from, for instance, biologically replicated hybrid crosses who possess a specific trait linked to a specific pattern of gene expression, inherited from one of the parents. A single sample taken from a pool can be used as a representative value for a treatment and it reduces the cost of the analysis procedure. On the other hand, if a statistical analysis of the variance in a pool is important for the question of a researcher, then sub pools can be analysed from the bulk pool in order to obtain the spread of whatever analysed in the bulk pool. This spread or variance can be used further for different statistical tests. Pak choi cv. 'Shanghai' was used to indicate the status of available elements when grown in T0, T1 and T2. Pak choi is a rapid crop with a high rate of leaf production during its exponential stage of growth which provides suited conditions for frequent tissue sampling.

Leaf tissues from each treatment were sampled, pooled and analysed for total N concentration in three occasions (week 2, 4 and 5) over a cultivation period of six weeks. This step in the analysis was done to compare the levels with the plant sap

Plant sap N and P concentration were analysed from the oldest and less deficient leaves in the same three occasions as for the leaf tissues.

Three days prior to harvest, samples of all treatments were collected for measurements of fresh weight and dry weight to test $H_{0(2)}$ (see the hypothesis section).

Dry root and leaf material from these samples were analysed for total N concentration.

Soil samples were taken postharvest following the same procedure as the initial soil sample.

Summary of experimental details & data & statistics

In this study, 22 Pak choi plants were grown in each plot, divided into two rows of plants with 11 plants per row. Repeating, each treatment had 10 replicated plots which gives 22 plants x 10 plots = 220 individuals per treatment which is 660 plants used in a total of three treatments: T0, T1 and T2. All these treatments with corresponding plots were totally randomised over the experimental area in the field. The amount of individual plants in each plot (22) determines the degree of freedom in the statistical computation which will reduce statistical errors that can arise from differences in the soil at plot level. Samples of plant tissues and incubated soil were collected during three occasions: week 2, 4 and 5 post field transplantation of plug plants. All plants experienced the same duration and volume of watering since drip irrigation was installed. Further on, three days prior to the final harvest plants were sampled to measure fresh weight and dry weight. The sampling was performed by computing an average of fresh weight and dry weight from two randomly selected plants taken from each plot. Thus, a total of 20 randomly selected plants were taken per treatment which generated 10 averaged observations per treatment (collected from 10 plots). These 10 averages (derived from each treatment) were statistically tested against each other using Student's t-test, in addition to an analysis using a generalised linear model ('glm') in R (version 3.5.3). The 'glm' output parameters were used for a post hoc test (pairwise comparison according to Tukey's HSD) using the R package 'emmeans'. The final harvest was carried out three days after the sampling and measurements of fresh weight and dry weight. Final harvest data was statistically tested according to the same procedure described for the FW and for the DW measurement. Moreover, binary counts (1 or 0) were taken on *visible* inflorescences for every plant in each treatment (600 individuals in total). The inflorescence counts data was then analysed with 'glm' but unlike for the 'glm' of yield data (continuous data), an argument for discrete data (or 'Binomial' distribution) was specified.

Results

All the results of pooled plant tissues and pooled soil samples collected from T0, T1 and T2 in this experiment should be interpreted as indications which reflect the nutritional status of soil and plants, in response to different fertilisers.

Nitrogen levels in leaf sap & leaf tissues & soil

Results of leaf tissue total N concentration (see figure 1) shows similar trends compared to the trends of plant sap N concentration shown in Fig. 2, except for the last occasion of sampling in Fig. 2 (28/05). The first observations of N levels in Fig. 1 and Fig. 2 (07/05) shows high relative accessibility to N for plants grown in T2 compared with plants grown in T1 and T0. Application of the BRD fertiliser in T2 was carried out one week prior to the first date of observation (07/05).

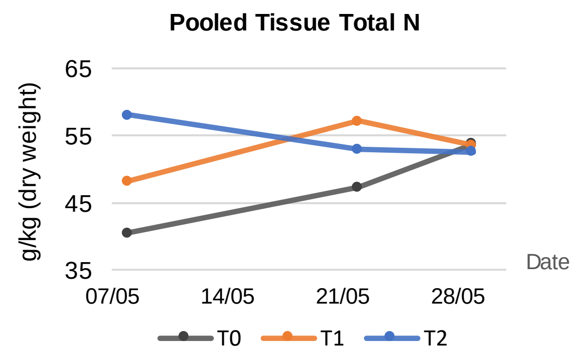

Figure 1 Leaf Tissue Total Nitrogen Concentration, in the youngest fully developed leaves of Pak choi plants sampled from each treatment on three occasions. Each data point shows the concentration of total N from a pool of approx. 100 leaves.

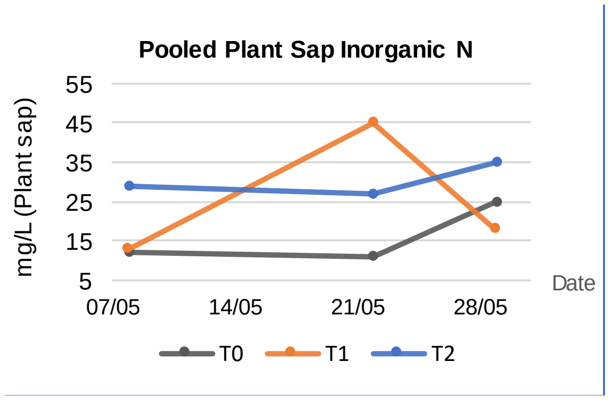

Figure 2 Plant Sap Inorganic Nitrogen Concentration, in the oldest and less defected leaves of Pak choi plants sampled from each treatment. Each data point shows the concentration of total N from a pool of approx. 100 leaves.

Nitrate content

According to the Swedish National Food Agency, nitrate N has a threshold concentration in unspecified salat (3000 – 5000 mg/kg), ruccola salat (7000 mg/kg) and spinach (3500 mg/kg) (NFA 2017 – table 4). No treshhold value for Pak choi is given in NFA (2017).

The results of nitrate N concentration in plant sap of plants grown in T0, T1 and T2 are presented in Fig. 9, given mg/L. All concentrations of plant sap nitrate N (Fig. 9) observed for the given weeks in this study shows lower values than the lowest treshhold value for the leafy vegetables listed in NFA (2017).

The plant sap levels shown in Fig. 9 triggered further interest to analyse nitrate N in dry leaf tissues. Samples were taken from the same leaf pool analysed for total N (see figure 4). These samples were sent to the laboratory in order to confirm the nitrate N concentration. The resulting concentratons of nitrate in dry laef tissues were: T0 nitrate N = 478 mg/kg, T1 nitrate N = 440 mg/kg, T2 nitrate N = 343 mg/kg. The highest concentration of nitrate N was found in T0, having the lowest relative concentration of ammonium N compared to T1 and T2. The concentration of nitrate N in T0 are still approx. six times lower than the lowest treshhold value listed for nitrate N in unspecified sallat (*Lactusa sativa* L.) and approx. 14 times lower than the treshhold value listed for ruccola (*Eruca sativa* Mill.). Ruccola and spinach belongs to the same family as Pak choi (*Brassicaceae*).

Figure 9 Plant sap nitrate concentrations for all weeks of sampling, (A) shows the concentration of ammonium N in leaf tissues sampled in week 2, 4 and 5 post field transplantation. (B) shows the nitrate N concentration for the given weeks. No samples were taken in week 3.

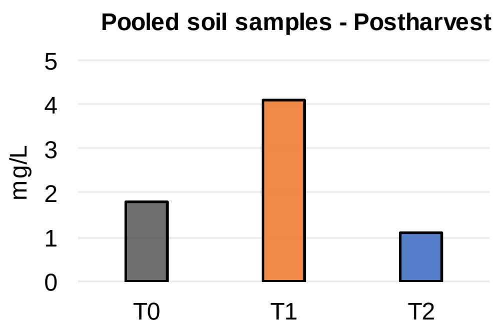

Figure 3 Soil Inorganic Nitrogen Concentration, each bar indicates the concentration of inorganic-N (ammonium + nitrate) found in the soil for each treatment postharvest.

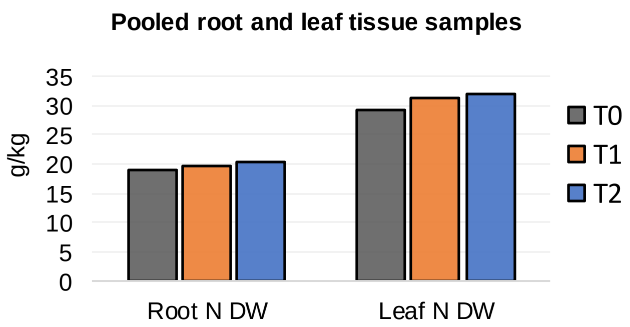

Figure 4 Plant Tissue Total Nitrogen Concentration, in sampled root and leaf tissues three days prior to the final harvest.

Dry & fresh weight

Leaf and root tissues were sampled from T0, T1 and T2 to measure fresh weight (FW) and dry weight (DW) 43 days post field transplantation (see figure 5) and total N concentration was measured for the same samples (see figure 4) where relatively small differences are shown for the root N concentration in DW. The roots of plants grown in T2 had significantly higher DW compared to both T0 and T1 (figure 5). In Fig. 4, DW and FW of sampled root and leaf tissues show similar concentrations of total N for the plants grown in T2 and T1. However, the difference in soil concentration indicates higher accessibility to inorganic nitrogen for plants grown in T1 compared to plants grown in T2 and T0. This concentration did not reflect any significant output on the final harvest (see figure 6) and plant sap concentration of inorganic N (see figure 2) was contractively shown to be the lowest in T1 samples (see discussion).

A complementary two sample t-test (Welch) testing the root DW and leaf DW from T1 and T2 samples resulted in p = 0,02 for the comparison of root DW, and p = 0,63 for the comparison of leaf DW. Thus, the alternative hypothesis $H_{0(2)}$ is true for the comparison of T1 and T2 root DW according to results gained from the 'glm' analysis with post hoc test in 'emmeans' according to Tukey's HSD, and results gained from the t-test (Welch).

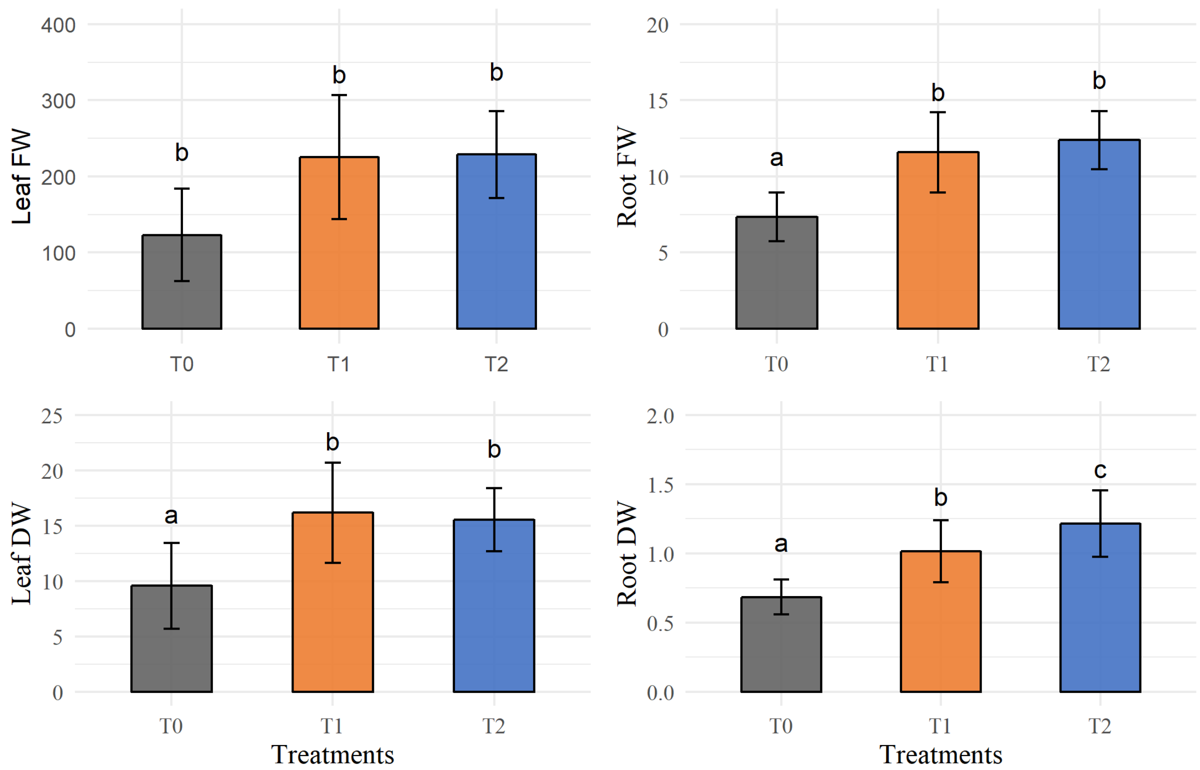

Figure 5 Fresh Weight (FW) and Dry weight (DW), each bar indicates the sampled mean of FW and DW three days prior to final harvest. Letters describe the statistical grouping; all bars within each subplot that are not sharing the same letter are significantly different from each other according to the 'glm' analysis followed by the post hoc test in 'emmeans' (Tukey HSD).

Final harvest

The final harvest (Fig. 6) was measured for yield 46 days post field transplantation. No statistical difference in means of yield were found between T1 and T2 according to the 'glm' analysis with post hoc test in 'emmeans' (Tukey HSD). Thus, $H_{0(1)}$ is true regarding the comparison of means of final harvest data for T1 and T2. Table S2 in supplementary material shows the detailed 'glm' parameters for the final harvest.

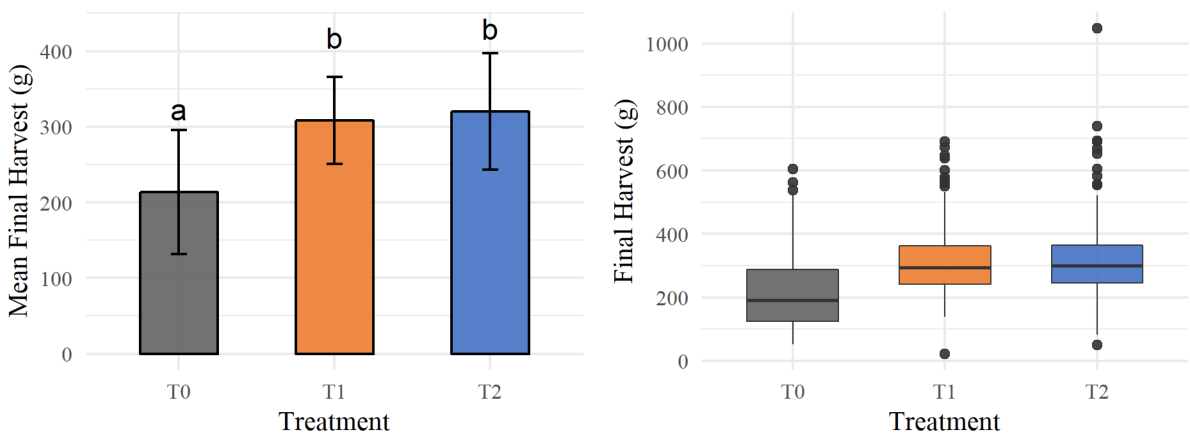

Figure 6 Final Harvest Yield Data (right) & Final Harvest Mean Yield (left), each bar (left) shows the mean value of 200 observations harvested from its respective treatment. Each boxplot (right) manifests the same 200 observations sampled from each treatment. Letters (left) describes the statistical grouping of the mean values; all means that are not sharing the same letter are significantly different from each other according to the 'glm' analysis with post hoc test in 'emmeans' (Tukey HSD). (See table S2 for statistical parameters).

Phosphorus - plant & soil levels

Highest concentration of postharvest soil remaining phosphorus was found in T2 (0,03 mg/L) compared to T1 (0,025 mg/L) and T0 (0,019 mg/L). Soil remaining concentration of phosphorus in T2 indicates redundant levels in relation to the plant sap concentration found in T2 (see Fig. 7). The values for plant sap concentration presented with week numbers (2, 4 and 5) in Fig. 7 were averaged and divided by the postharvest soil remaining phosphorus concentration for each treatment and the ratios are presented in Fig. 8.

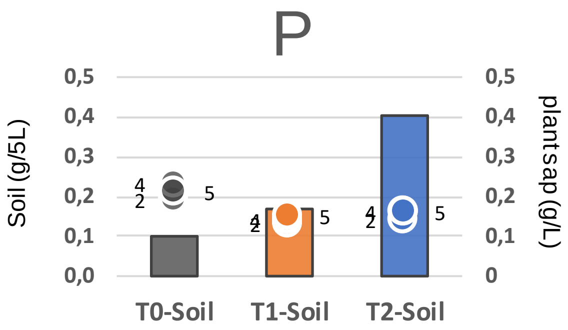

Figure 7 Plant sap concentration of phosphorus in relation to cultivation starting concentration in soil, plant sap concentration is indicated with data points and graded with the right y-axis. Assigned numbers (2, 4 and 5) to each data point indicates the week in which the sample was taken post filed transplantation. Bars indicate soil concentration graded with the left y-axis.

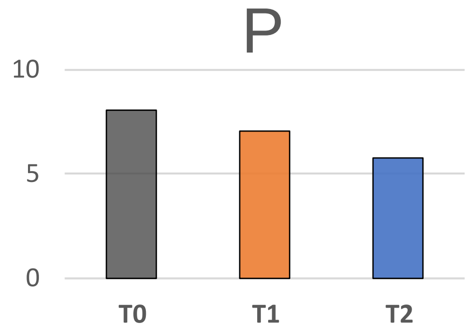

Figure 8 Ratio of averaged plant sap phosphorus to postharvest soil remaining phosphorus, computed by taking the mean concentration of plant sap phosphorus from plants grown in each treatment and divide those means with the postharvest soil remaining P concentration of each treatment. The highest difference of this sap-to-soil ratio was shown for T0 (plants grown in control soil without added fertilisers). This shows that T0 plants grown in the lowest relative soil concentration of P were able to accumulate the highest relative sap concentration of P (see Discussion).

Discussion

The growth and development of plants can be demonstrated with an S-shaped slope (an 'S-curve') where x = time and y = growth rate. For most pre-cultured seedlings, the log-phase of growth (i.e. the lower tail of the 'S-curve') passes in the nurseries during the pre-cultivation of seedling. Plug plants of rapid leafy vegetables requires instant access to nutrition in connection with field transplantation to ensure maximum growth and development (yield). In any form of plant production farmers and urban producers should take material specific nutritional release rates and batch specific concentrations into consideration and follow advice given by professionals from the agricultural sector. Appropriate fertiliser management saves resources, maximizes crop productivity (yield) and reduces pollution of soil remaining fertilisers. Regardless of the system (conventional/integrated or organic) N and P percolation is mainly important to reduce due to the impact on eutrophication in watercourses, lakes and further downstream in the oceans. Organic matter can be sensitive for leakage of both N and P since their release rate varies over the season depending on several factors given in the introduction (for instance the ratio of C/N) which differs among organic materials. The challenging part is to apply the fertilisers with the ambition to supply maximised plant growth during the accelerated stage of growth in connection to field transplantation of precultured leafy vegetables like Pak choi.

The ACM assayed in T2 could possibly had affected the accessibility to the proportion of inorganic N added from the BRD through immobilisation (beyond plant absorption) indicated in Fig. 1 with a descending trend of total N concentration observed in T2. In contrast to T2, T1 shows an increasing trend of total N concentration (Fig. 1) which can depend on slow disintegration of PSW in soil after application. All treatments ended up showing similar concentrations of total N in the last date of sampling (figure 1 – 28/05). Further, the last date of sampling of plant sap (figure 2 – 28/05) showed the lowest N concentration in T1 and the highest concentration in T2. This particular relationship of plant sap concentration between T1 and T2 is mediated as contradictive since the *lowest* concentration of postharvest soil inorganic N was found in T2, and the *highest* concentration of postharvest soil inorganic N was found in T1, see Fig. 3. A more reasonable scenario would have been that the plant sap concentration actually reflected the concentration in soil, high sap concentration mirrors high soil accessibility which is shown in Fig. 1 and Fig. 2 for the first two occasions of sampling

(07/05 and 21/05) but not the last (28/05). This pattern is communicated here as a reverse soil-sap concentration relationship which most likely arised from bias. The low inorganic levels of N found in the soil of T2 postharvest indicates that the nitrogen was either effectively utilised by the plants along with some immobilisation and/or that a part of the N got lost through gasification events. The immobilisation, however, depends on the summarised C/N of ACM and the present C/N in soil post fertiliser application. In fact, ACM have an approximated C/N = 20 (SJV 2018, see table 3) which is considered relatively high compared to BRD and PSW.

The final harvest (Fig. 6) did not differ between T1 and T2 according to the 'glm' analysis with post hoc test in 'emmeans' (Tukey´s HSD) and the two-sided t-test (Welch) see table S2, S3, S4. The low difference in final harvest comparing T1 and T2 can also be interpreted as 3.9% higher total yield summary in T2, over T1. Moreover, the difference of added N content in T1 and T2 can be interpreted as 23% more nitrogen in T2. To compare, a 3.9% difference in yield gain compared to a 23% difference in N application between T2 to T1 (see table S1 – summary of nitrogen in treatment 1 compared to treatment 2) indicate that N can be lowered in T2 without losing a significant amount of yield. A common procedure to implement after a pilot study (like this work) is to optimise application by testing gradients of fertiliser concentrations with further statistical approaches.

The results of this study indicates that the concentration of phosphorus can be lowered in T2 since the plant sap levels of phosphorus reflects that even if the soil phosphorus concentration is 2,4 folds higher in T2 compared to T1, and 4 folds higher compared to T0 (see bars in figure 7), plant sap concentrations of P in T1 and T2 stayed similar over time and the *highest concentration* was surprisingly found in T0 plant sap, surprisingly, since T0 had the *lowest soil phosphorus concentration*, Fig. 7. Grow catch crops is a sustainable way to prevent the loss of negatively charged anions like nitrates and phosphates when these are present in redundant levels in soil postharvest.

The general growth/development of T2 went somewhat faster during the first three weeks. This was mainly observable on the number of leaves counted ones on intact plants from all treatments during week three; data not saved since not intended to be measured at first place, however, a response variable which indicated the momentary growth rate. Moreover, an observation of visible inflorescences was also performed on *all* 600 plants (T1, T2, T3) in connection with final harvest. It turned out that T2 had a significant higher number of inflorescence counts compared to T1 and T0 which indicates faster development as a result of the fertilisers applied in T2, see table S5 in supplementary material for 'glm' parameters of inflorescence counts. As a matter of fact, plants in T2 could have been harvested at least a week earlier in comparison to T1 and T0. Worth to discuss here is: was the enhanced flowering process in T2 dependent on the 3.9% difference in N concentration compared to T1 – most likely not since the control with very much lower N concentration did not differed from T1 in terms of inflorescence counts, see table S5. In addition, the growth of Pak choi has shown to be significantly enhanced in soil treated with cattle manure (Watanabe *et al*. 2012). The major fact communicated is the potential for earlier harvest of plants grown in T2.

Conclusions

This work concluded that using ACM as the primary source of P indicates redundancy revealed by the ratio of soil P and averaged plant sap P (figure 8) when applying the ACM as the primary source of K. In case of balancing the ACM with respect to P, a shortage of K will appear () which can be supplemented with an alternative K-source, for instance 'Kalimagnesia' which content of magnesium (Mg) moreover complements the low level of Mg found in the BRD. This reasoning is suggested for this particular fertiliser regime suited to supply Pak choi with non-redundant levels of P to avoid over fertilisation.

The PSW is concluded to be assayed in a new experiment, but together with the BRD as a supplementary source of N. The BRD could in other words be added together with the PSW (NPK 6-3-12) with the same objective as adding it together with the ACM (like in this experiment) – to apply a high concentration of inorganic nitrogen in connection to field transplantation in order to provide instant access of inorganic N to plug plants. Moreover, this procedure is suggested to be performed using a gradient of concentration of BRD to avoid over exposure and loss of N because the C/N is lower in the soil when testing the PSW as a nutritional foundation instead of the ACM like in this study. This modification is concluded due to the high soil concentration of inorganic nitrogen found in T1 postharvest; which could be lowered in case of lowering the starting amount of N application from the PSW and have this difference of N supplemented instead with the BRD. In other words, the BRD can compensate for the low (early season) N release of the pelleted fertilisers (PSW) which in this study is indicated with a relative low concentration of plant sap N in the first occasion of tissue analysis (see figure 1 and figure 2 – 07/05). The *dry* and compressed organic waste in the PSW require some soil heat and moisture for effective dissolution in early spring and this affects the availability of N from PSW. Anyhow, the amount of SOM is suggested, primary, to determine which of these two choices (PSW or ACM) to add in conjunction to the BRD – ACM is added in case of low SOM and PSW is added if the SOM is in balance.

The ACM together with one single application of BRD in T2 leaves less inorganic nitrogen in soil postharvest (see figure 3). Plants in T2 accumulates a somewhat higher concentration of (pooled) total N in roots and leaves, in contrast to T1.

The level of fertilisers (ratios and concentrations) is concluded to be lowered in both of the treatments for further experiments, with the aim to compare the yield with plant tissue concentrations of different macro elements in a restricted manner (or with less accessibility to the elements as a consequence of assaying concentration gradients in the soil). With a stronger budget, such a project can be conducted through the analysis of sub-pools to generate data for at least five observations per treatment. This repeated measurement will be sufficient if the aim is to apply comparative statistics to test sample variance.

Extensive levels of fertilisers will not increase the yield, rather cause different issues like eutrophication and unnecessary loss of natural resources, e.g. P. Peak phosphorus is one example of a projection of the limitation of phosphorus in agriculture which soon will become an important area of research *on global level*.

Thank you for reading this thesis.

References

Ahmed, E. (2015). Microbe-mineral interactions in soil: Investigation of biogenic chelators, microenvironments and weathering processes. *Meddelanden från Stockholms Universitets Institution för Geologiska Vetenskaper*. Stockholm University, Department of Geological Sciences. https://www.diva-portal.org/smash/record.jsf?pid=diva2%3A796173&dswid=-1237

Albelda-Berenguer, M., Monachon, M., Joseph, E. (2019). Siderophores: From natural roles to potential applications. *Advances in applied microbiology*, vol. 106, pp. 193–225. DOI: https://doi.org/10.1016/bs.aambs.2018.12.001

Chen, L. & Bush, D. R. (1997). LHT1, a Lysine- and Histidine-Specific Amino Acid Transporter in Arabidopsis. *Plant physiology (Bethesda)*, vol. 115 (3), pp. 1127–1134. American Society of Plant Physiologists

Deng, S., Wipf, H.M.-L., Pierroz, G., Raab, T.K., Khanna, R. & Coleman-Derr, D. (2019). A Plant Growth-Promoting Microbial Soil Amendment Dynamically Alters the Strawberry Root Bacterial Microbiome. *Scientific reports*, vol. 9 (1), pp. 17677–17677. DOI: https://doi.org/10.1038/s41598-019-53623-2

Dordas, C. (2008). Role of nutrients in controlling plant diseases in sustainable agriculture. A review. *Agronomy for Sustainable Development volume.* 28**,** 33–46. https://doi.org/10.1051/agro:2007051

Fukui, R. (2003). Suppression of Soilborne Plant Pathogens through Community Evolution of Soil Microorganisms. *Microbes and Environments*, vol. 18 (1), pp. 1–9 Miyagi: Japan Science and Technology Agency. DOI: https://doi.org/10.1264/jsme2.18.1

Hirner A., Ladwig F., Stransky H., Okumoto S., Keinath M., Harms A., Frommer W.B. & Koch W. (2006). Arabidopsis LHT1 Is a High-Affinity Transporter for Cellular Amino Acid Uptake in Both Root Epidermis and Leaf Mesophyll. *The Plant cell*, vol. 18 (8), pp. 1931–1946. DOI: 10.1105/tpc.106.041012

Jämtgård S. (2010). *The Occurrence of Amino Acids in Agricultural Soil and their Uptake by Plants*. Diss. Swedish University of Agricultural Science. Umeå: Arkitektkopia AB 2010

Lauber, C.L., Hamady, M., Knight, R. & Fierer, N. (2009). Pyrosequencing-Based Assessment of Soil pH as a Predictor of Soil Bacterial Community Structure at the Continental Scale. *Applied and Environmental Microbiology*, vol. 75 (15), pp. 5111–5120. DOI: 10.1128/AEM.00335-09

Liao, J., Yun, L. & Danfeng, H. (2018). Organic Farming Improves Soil Microbial Abundance and Diversity under Greenhouse Condition: A Case Study in Shanghai (Eastern China). *Sustainability* (Basel, Switzerland), vol. 10 (10) MDPI AG. DOI: https://doi.org/10.3390/su10103825

Sullivan, D. M. (2020). Estimating Plant-available Nitrogen from Manure. *Oregon State University*, EM 8954-E Revised July 2020. https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&ved=2ahUKEwj8lr3Dt-HrAhWLl4sKHUnBBR4QFjABegQIAhAB&url=https%3A%2F%2Fcatalog.extension.oregonstate.

edu%2Fsites%2Fcatalog%2Ffiles%2Fproject%2Fpdf%2Fem8954.pdf&usg=AOvVaw0Js5GLvlG FMCQWNeTd718U [2020 09 11]

Steffens, D., Hütsch, B., Eschholz, T., Lošák, T. & Schubert, S. (2005). Water logging may inhibit plant growth primarily by nutrient deficiency rather than nutrient toxicity. *Plant, soil and environment*, vol. 51 (No. 12), pp. 545–552

SCB (2019). *Ekologisk växtodling 2019*. JO13 SM 2001 - Ekologisk växtodling. Statistikmyndigheten (SCB) [2020 05 20]

SJV (2016). *Gödselmedel för ekologisk odling 2016.* Specialgödselmedel och stallgödsel (Version 2015-12-18), Uppsala. Statens Jordbruksverk (SJV). https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&ved=2ahUKEwj_rKba_qnrAhVnx4sKHWETC_sQFjACegQIBRAB&url=http%3A%2F%2Fwww2.jordbruksverket.se%2Fdownload%2F18.732fab17151c51395339007a%2F1450787304613%2Fovr376.pdf&usg=AOvVaw1n0q7ZDaJN5-eD1C_AfKz5 [2020 08 20]

Schlüter, S., Henjes, S., Zawallich, J., Bergaust, L., Horn, M., Ippisch, O., Vogel, H.-J. & Dörsch, P. (2018). Denitrification in Soil Aggregate Analogues-Effect of Aggregate Size and Oxygen Diffusion. *Frontiers in environmental science*, vol. 6 Frontiers Media SA. **DOI:** 10.3389/fenvs.2018.00017.

SJV (2018). *Rekommendationer för gödsling och kalkning, 2019.* Jordbruksinformation 18. Statens Jordbruksverk (SJV). https://webbutiken.jordbruksverket.se/sv/artiklar/jo1818.html [2020 08 20]

Watanabe T., Takumi E., Michihiko S., Miwa O., Takuro S. & Mitsuru O. (2012). DIFFERENCES IN ACQUISITION OF ORGANIC NITROGEN IN SOILS BETWEEN BOK CHOY AND TOMATO. *Journal of Plant Nutrition*, 35:10, pp. 1468-1476. DOI: 10.1080/01904167.2012.689907

Supplementary material

Table S1 Summary of all fertilisers which was added to T1 and T2. The amount of nitrate in the ACM analysed according to QuAAtro (post field application) is indicated with +158, see column: N. This amount of nitrate lead to an unbalanced summary concentration of N between T1 and T2.

Fertilisers	T1	T2	Added (g/plant)	N	P	K	Ca	Mg	S
ACM (NPK)		X	**177.8**	294 + 158	390	814		266	248
BRD (NPK)		X	**57.8**	235	14	104		3	17
PSW (NPK)	X		**6.8**	302	163	814		7	495
PSW (N)	X		**1.9**	228	6				43
Kieserit (Mg + S)	X		**17.4**					262	349
		X	**31.1**					467	623
Dolomit (Mg)	X		**7.8**					934	
		X	**3.9**					467	
Summary T1 (mg/plant)				530	169	814		1203	887
Summary T2 (mg/plant)				687	404	918		1203	888

Table S2, 'glm' parameters of final harvest based on 200 obs. of yield data per treatment.

Contrast	Estimate	SE	Df	z-ratio	p-value
T0 – T1	95	11.6	infinity	8.151	<0.001***
T0 – T2	107	11.6	Infinity	9.183	<0.001***
T1 – T2	12	11.6	infinity	1.031	**0.5570**

Table S4, two sample t-test (Welch) assuming equal variances of the final harvest.

Contrast	df	t-value	p-value
T1 – T0	18	2.9973	0.00773**
T2 – T0	18	3.0106	0.00751**
T2 – T1	18	0.3957	**0.679**

Table S3, yield parameters given in grams (g) and kilograms (kg) as indicated.

Treatments	Mean yield 200 obs. ± SD (g)	Summed yield 200 obs. (kg)
T0	213 ± 82	42.67
T1	308 ± 57.6	61.66
T2	320 ± 76.8	64.06

Table S5, 'glm' parameters where 'contrast' indicates the comparison (in pairs) of inflorescence counts based on binomial data (1,0) observed for all 200 obs. in each treatment. The contrast of T0 to T1 reveals no effect on inflorescence count which indicates that the fertiliser treatment in T1 had no effect on enhanced flowering.

Contrast	Estimate	SE	df	z-ratio	p-value
T0 – T1	0.171	0.338	infinity	0.505	0.8688
T0 – T2	1.242	0.296	Infinity	4.200	0.0001***
T1 – T2	1.071	0.282	infinity	3.797	0.0004***